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Evaluation of Tolerance of Several Corn Genotypes (*Zea mays* L.) to Salinity Stress at the Germination Stage

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Abstract

Corn (*Zea mays* L.) is a vital food crop with the potential to enhance food supply and support other sectors, such as livestock production. This study aims to determine the level of salinity stress that can be utilized to select maize genotypes tolerant to such stress. The research was conducted in the greenhouse of the Maros Cereal Research Center (Balitsereal) starting in February. The experimental design employed a split-plot arrangement consisting of two factors. The main plot consisted of five levels of NaCl concentration: no salinity stress and concentrations of 30, 60, 90, and 120 mM. The subplot included eight maize genotypes. Each treatment combination was replicated three times. Corn seeds were germinated for five days in petri dishes lined with germination paper, with ten seeds per dish. Afterward, the seeds were treated with NaCl and distilled water. The germinated seeds (after 5 days) were then transferred to the next germination medium, specifically styrofoam. At the germination stage, the tolerance selection of several corn genotypes under salinity stress revealed that NaCl concentration significantly affected plumule length, root length, and the number of roots. As the NaCl concentration increased, plumule length, root length, and the number of roots decreased. Additionally, genotype significantly impacted root length, the number of roots, and the percentage of seed growth. The results of the variance analysis indicated that there was no interaction between NaCl concentration and genotype at the germination stage.

Keywords: Corn, Genotype, NaCl, Salinity, Tolerance

1. Introduction

Corn (*Zea mays* L.) is a vital food crop, serving as a substitute for rice for a portion of the Indonesian population. Additionally, corn is a strategic commodity that significantly influences economic stability. This importance is driven by the rising demand for corn, which is needed for food production, animal feed, and industrial raw materials. Furthermore, by-products such as stems, leaves, and husks can be utilized as organic mulch or compost fertilizer. With advancements in knowledge and technology, corn is also being developed as a source of energy, as it is one of the plants that can produce bioethanol in substantial quantities.

Corn production in Indonesia from 2011 to 2015 experienced fluctuations. The lowest production level, recorded at 17.64 million tons, occurred in 2011. However, production increased to 19.0 million tons in 2014, and in 2015, the growth rate rose by 3.18%, reaching 19.61 million tons (BPS, 2015). The Government continues to promote initiatives to significantly increase corn production, which is driven by the growing population and the industry's expanding needs.

The Government continuously works to increase corn production along with the increasing population. One of the obstacles in increasing corn production is the decreasing fertile lands suitable for corn planting conditions as a result of land conversion into industrial and residential areas, so that productive agricultural lands are decreasing. The Ministry of Agriculture estimates that the conversion of agricultural land to the non-agricultural sector reacheS47 thousand hectares per year (Nasution, 2006). Suppose fertile land has been converted for other needs. In that case, another option is to work on marginal lands with various environmental stress problems, including land affected by saline.

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Environmental stress is a factor that inhibits plant growth. Among the various environmental stresses, salinity is one of the most common stresses (Gedoan et al., 2004). In Indonesia, there are around 39.4 million hectares of saline land. In South Sulawesi, approximately 1 million hectares of land cannot be planted due to salinity problems, especially in coastal areas such as Jeneponto, Pangkep, Bantaeng, Selayar, and Barru Regencies. Salinity increasingly receives attention in agriculture because it causes plant stress (Nugraheni et al., 2003). Therefore, varieties resistant to high salinity conditions are needed so that marginal land is expected to be more conducive to increasing production.

Since corn plants were developed in Indonesia, various varieties have been produced, both hybrid and composite varieties originating from within the country and from introductions. However, not all varieties that have been developed have good tolerance to stressful environments, especially on saline land.

Several studies have evaluated the effect of salinity on maize growth, but most focus on the vegetative and generative phases (Latif et al., 2015; Hussain et al., 2019). The germination stage is a critical phase that greatly determines the success of plants in dealing with stress in the next phase (Motsa et al., 2017). In addition, previous studies generally used one or two maize varieties, so comparative data between wider genotypes is still limited (Ahmad et al., 2021). Not many studies specifically examine the differences in salinity tolerance in various local and introduced maize genotypes during the germination phase.

This study aims to develop salinity stress-tolerant corn varieties that need to be carried out through breeding activities to obtain superior varieties with high yield potential and wide adaptability, including the assembly of new superior varieties that are adaptive to saline land. This effort is significant in maintaining food security. The initial step to obtain saline-tolerant corn varieties is to conduct resistance tests on several existing varieties to assess their resistance levels based on their NaCl concentration. Based on these tests, the varieties that have the best resistance to salinity conditions can be determined.

2. Material and Methods

2.1. Time and implementation

Seed germination was conducted from February to March 2017 at the Laboratory of the Cereal Crops Research Center (Balitsereal) Maros. Jl. DR. Ratulangi No.274, Allepolea, Kec. Lau, Maros Regency, South Sulawesi 90512 4.9822° S, 119.5743° E, altitude 27.2 meters above sea level.

2.2. Materials and tools

Germination rate: the materials used for germination are 8 corn genotypes, NaCl, and Gandasil D fertilizer. The tools used are Petri dishes, styrofoam, rulers, trays, measuring cups, Mohr pipettes, analytical scales, magnetic stirrers, meters, stationery, callipers, label paper, plastic and rulers.

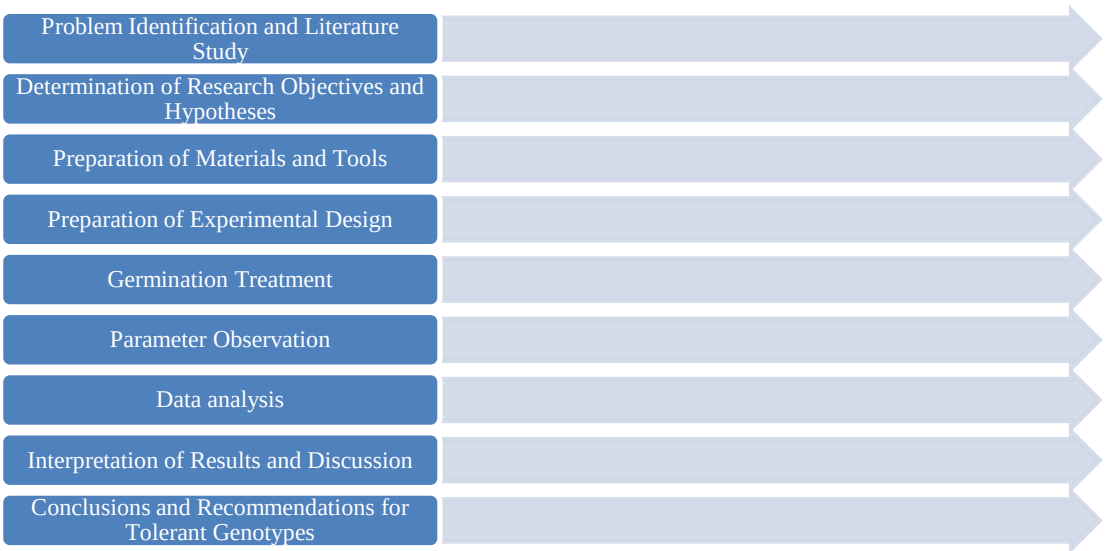

Figure 1. Research flow diagram

2.3. Experimental method

This study was arranged based on a split-plot design (RPT) of two factors. The main plot (PU) is the NaCl concentration consisting of 5 levels, namely without salinity stress (S0), 30 mM concentration (S1), 60 mM concentration (S2), 90 mM concentration (S3), 120 mM concentration (S4) and the sub-plot (AP) is the corn plant genotype consisting of 8 genotypes, namely g1, g2, g3, g4, g5, g6, g7, g8.

Corn seeds were germinated for 5 days in a petri dish lined with 10 germination papers. After that, the seeds were treated with NaCl solution mixed with distilled water. The

germinated seeds (after 5 days) were transferred to the next germination medium, namely styrofoam. Before being assigned to styrofoam, a solution storage container was prepared first. The styrofoam was formed into a rectangle and given 10 holes for each genotype so that 80 holes would be used to plant the sprouts obtained from the previous germination stage. The styrofoam was put into a container containing 1000 mL of distilled water that had been mixed with NaCl solution according to the level of salinity stress, namely 0 mM, 30 mM, 60 mM, 90 mM and 120 mM and left for 10 days, then transferred to a polybag.

2.4. Data analysis

The summarized data will be analyzed for variance. Analysis of variance to determine the differences in response between genotypes and salinity stress levels. If the analysis of variance shows that the genotype response and salinity stress level have a significant effect, then it is continued with a BNT further test at the 95% level. Using Microsoft Excel for Microsoft 365 application.

3. Results and Discussion

3.1. Percentage of seed growth (%)

The results of the observations regarding the percentage of seed growth and the analysis of variance are presented in Table 1. The analysis of variance indicates that the genotype treatment had a highly significant effect on the percentage of seed growth. In contrast, the salinity treatment and the interaction between the two factors did not have a considerable impact.

Table 1. Percentage of seed growth (%) in various genotypes.

Salinity	Genotype (G)							
	g1	g2	g3	g4	g5	g6	g7	g8
(control)	90.00	66.67	90.00	100.00	83.33	70.00	83.33	93.33 ± 2.0
(30 mM)	100.00	80.00	90.00	86.67	63.33	73.33	95.00	95.00 ± 3.5
(60 mM)	96.67	83.33	93.33	90.00	80.00	73.33	96.67	80.00 ± 2.1
(90 mM)	90.00	83.33	90.00	90.00	90.00	66.67	66.67	83.33 ± 2.7
(120 mM)	100.00	73.33	96.67	100.00	83.33	73.33	96.67	86.67 ± 2.0
Average	95.33 [a]	77.33 [ab]	92.00 [ab]	93.33 [ab]	80.00 [ab]	71.33 [b]	87.67 [ab]	87.67 ± 2.0 [ab]
NP BNTg 0.01				22.39				

Description: Numbers followed by the same letter in the row (a,b) mean that they are not significantly different in the BNT test at level α0.01.

Based on the results of the BNT test at level α0.01 (Table 1), it shows that g 1 shows a good percentage of seed growth, namely 95.33% and is significantly different from g6, while g 6 shows a poor percentage of seed growth, namely 71.33%.

The results of observations of plumule length and its variance analysis are presented in Table 2. The variance analysis shows that salinity treatment significantly affects plumule length, while genotype and the interaction between genotype and salinity have no significant effect.

3.2. Plumule length (cm)

Table 2. Average plumule length (cm) at various levels of salinity stress at 5 HST.

Salinity	Genotype (G)									
	g1	g2	g3	g4	g5	g6	g7	g8	Average	NP BNTS0.05
(control)	4.79	3.35	2.86	3.26	4.00	3.48	3.15	3.80	3.59 ± 0.2 [a]	
(30 mM)	3.73	1.90	3.04	3.10	2.44	2.28	1.84	2.33	2.58 ± 0.3 [b]	
(60 mM)	2.57	2.13	1.33	2.49	3.10	2.08	1.86	1.83	2.17 ± 0.3 [c]	0.13
(90 mM)	1.61	1.78	1.74	2.10	1.99	1.85	1.79	2.25	1.89 ± 0.4 [d]	
(120 mM)	1.83	1.32	1.51	2.69	2.33	1.09	1.99	1.51	1.78 ± 0.1 [d]	
Average	1.95	1.74	1.74	1.92	1.92	1.75	1.75	1.79		

Description: Numbers followed by the same letter in the same column (a,b, c,d) mean that they are not significantly different in the BNT test at the 0.01 level α.

Based on the results of the BNT test at level α0.01 (Table 2), it shows that without salinity stress (control), it produces an average of the longest plumule length, namely 3.59 cm and is very significantly different from the root length produced at all levels of salinity treatment, while the average of the shortest plumule length is made at stress s 5 (120 mM), namely 1.78 cm.

3.3. Root length (cm)

The results of observations of root length and its variance analysis are presented in Table 3. The variance analysis shows that salinity and genotype treatments have a significant effect. In contrast, the interaction between the two has no significant impact on the root length of corn plants.

Based on the results of the BNT test at level α0.01 (Table 3) showed that without salinity stress (control), it produced an average of the longest root length of 7.01 cm and was significantly different from the root length produced at all levels of salinity treatment. In comparison, treatment s 5 (120 mM) showed an average of the shortest root length of 4.01 cm. For genotypes, g 1 produced an average of the longest root length of 8.39 cm and was very significantly different from all genotypes tested, while g 2

and g3 showed an average of the shortest root length of 3.80 cm.

Table 3. Average length of seedling roots (cm) in various genotypes and salinity stress levels at 5 HST.

Salinity	Genotype (G)									
	g1	**g2**	**g3**	**g4**	**g5**	**g6**	**g7**	**g8**	**Average**	**NP BNT 0.05**
(control)	10.69	4.53	4.72	9.07	8.23	5.63	5.69	7.50	7.01 ± 0.6 [a]	
(30 mM)	10.55	3.80	4.73	6.73	3.15	3.82	4.27	4.37	5.18 ± 0.7 [b]	
(60 mM)	7.93	4.31	3.22	6.78	5.48	3.63	4.22	3.47	4.88 ± 0.9 [b]	0.38
(90 mM)	6.26	3.61	3.38	6.38	5.07	3.67	5.23	3.99	4.70 ± 0.7 [b]	
(120 mM)	6.50	2.74	2.94	5.07	4.57	2.60	4.05	3.63	4.01 ± 0.8 [c]	
Average	**8.39** [v]	3.80 [z]	3.80 [z]	6.81 [w]	5.30 [x]	3.87 [z]	4.70 [y]	4.60 [y]		
NP BNT 0.01				**0.42**						

Description: Numbers followed by the same letter in the row (a,b) or the same column (w,x,y,z) mean that they are not significantly different in the BNT test at the α0.01 level.

3.4. Number of roots

The results of observations of the number of roots and root variance analysis are presented in Table 4. The variance analysis shows that salinity and genotype treatments have a significant effect, while the interaction between the two has no significant impact on the length of corn plant roots.

Table 4. The average number of sprout roots in various genotypes and salinity stress levels at age 5 HST

Salinity	Genotype (G)								Average	NP BNTS0.05
	g1	**g2**	**g3**	**g4**	**g5**	**g6**	**g7**	**g8**		
(control)	8.07	6.87	8.15	8.48	7.28	6.12	5.82	5.02	6.98 ± 0.7 [a]	
(30 mM)	7.57	6.35	6.35	8.53	5.45	4.58	4.2 0	3.63	5.83 ± 0.4 [b]	
(60 mM)	8.83	7.96	6.52	7.60	5.25	4.53	6.42	4.6 0	6.46 ± 0.7 [a]	0.92
(90 mM)	6.17	5.58	7.48	5.1 0	5.42	4.83	5.18	4.68	5.56 ± 0.3 [b]	
(120 mM)	9.08	6.70	7.72	8.4 0	6.68	4.1 0	6.13	5.73	6.82 ± 0.3 [a]	
Average	**7.94** [v]	6.69 [w]	7.24 [v]	7.62 [v]	6.02 [x]	4.83 [z]	5.55 [y]	4.73 [z]		
NP BNTg 0.01				0.46						

Description: Numbers followed by the same letter on the same line (a, b) or in the same column (v,w,x,y,z) means there is no significant difference in the BNT test at levels α0.01 and 0.05.

Based on the BNT test levelα, 0.01 and 0.05 (Table 4) show that at S1 (without salinity stress), the highest average number of roots was produced, namely 6.98. and significantly different from the number of roots produced at all levels of salinity treatment except S3 and s 5, while S4 (90 mM) showed an average number of small roots, namely 5.56. For genotypes, g 1 produced growth with the highest average number of roots, namely 7.94, significantly different from the genotypes tested except g 2 and g 3. In contrast, g 8 showed an average number of small roots, 4.73.

The ratio between plumule length and root length (cm)

The results of observations of the ratio of plumule length and root length and the analysis of variance are presented in Table 1. The analysis of variance shows that the genotype treatment has a very significant effect on the percentage of seed growth. In contrast, the salinity treatment and the interaction between the two have no significant impact.

Table 5. The ratio between plumule length and average seedling root length (cm) in various genotypes at 5 years of age HST.

Salinity	Genotype (G)							
	g1	**g2**	**g3**	**g4**	**g5**	**g6**	**g7**	**g8**
(control)	0.44	0.85	0.62	0.36	0.50	0.61	0.55	0.50
(30 mM)	0.35	0.50	0.67	0.46	0.77	0.63	0.29	0.35
(60 mM)	0.33	0.50	0.43	0.37	0.55	0.57	0.44	0.52
(90 mM)	0.27	0.49	0.51	0.33	0.42	0.48	0.35	0.62
(120 mM)	0.28	0.71	0.51	0.53	0.58	0.42	0.49	0.42
Average	0.33 ± 0.1 [a]	0.61 ± 0.3 [b]	0.55 ± 0.2 [b]	0.41 ± 0.3 [ab]	0.56 ± 0.4 [b]	0.54 ± 0.2 [b]	0.42 ± 0.1 [ab]	0.48 ± 0.3 [ab]
NP BNTg 0.01				0.20				

Description: Numbers followed by the same letter in the same row (a,b) mean that there is no significant difference in the BNT test at level α0.01.

Based on the results of the BNT test levelα 0.01 (Table 5) show that g1 has the lowest ratio between plumule length and root length (0.33) and is significantly different from g2, g3 and g6.

The results of the variance analysis showed that the NaCl concentration treatment significantly affected the

length of the plumule, the length of the root, and the number of roots. The higher the NaCl concentration, the length of the plumule, the length of the root, and the number of roots will decrease successively as seen in TableS2, 3, and 4. This is thought to be due to NaCl poisoning (Na + and Cl -), which causes a decrease in the length of the plumule, the length of the root and the number of roots. The opinions of Mohammed (1989) and Khan (1997) stated a tendency to decrease the germination rate due to increased salinity. On the other hand, Heenan (1988) noted that the reduction in the germination rate at high salt levels was caused by osmotic pressure.

A decrease in germination characteristics can occur because the plants experience osmotic stress caused by an increase in the concentration of dissolved salts so that cell division and expansion at the root tips are inhibited; this condition will reduce the total number of roots formed in each treatment so that overall the number of roots and root length will decrease (Heenam, 1988)

According to Latuharhary et al. (2016), the decrease in plumule length (Table 2) and root length (Table 3) can be caused by limited water and organic matter supplies in the tissue due to the influence of salinity. The decrease in the amount of water causes cells to lose turgor, so that there is a tendency for the plasmalemma to detach from the cell wall (plasmolysis). In the cell elongation process, plants require an appropriate water balance because the strength of cell elongation results from turgor pressure. The presence of water will increase the turgor of the cell wall, which causes the cell wall to stretch and the bonds between the cell walls to weaken. This drives the cell walls and membranes to enlarge, so the lack of water availability will inhibit plant growth. In addition, the decrease in plumule length and root length is thought to be caused by the influence of osmotic stress, which makes it difficult for plants to absorb water and the toxic effect of Na and Cl ions due to the administration of NaCl so that cell division and enlargement are inhibited and plants will grow stunted.

The level of inhibition on plumule length, root length and number of roots that differ at each level of salinity treatment is visible at concentrations s2 (30 mM), s3 (60 mM), s4 (90 mM) and s5 (120 mM) compared to without NaCl administration. Inhibition of the root formation process is thought to be due to the accumulation of NaCl salt in the root environment. Higher salt accumulation will inhibit stronger root growth.

The presence of osmotic stress due to the influence of NaCl on the growth medium causes the growing cells to experience a lack of water. In water shortage conditions, cell enlargement will decrease due to low cell turgidity. Jumin (2002) stated that the loss of cell turgidity can stop cell growth (cell duplication and enlargement), thereby inhibiting plant growth.

The analysis of variance showed that the genotype treatment had a very significant effect on root length, number of roots, percentage of seed growth and the ratio between plumule length and root length, as seen in Table 1, Table 3, Table 4 and Table 5. It is suspected that the genotypes have different tolerances to salinity stress levels. Mostafavi (2011) stated differences in tolerance between genotypes at all salinity levels.

Salinity greatly influences genotypes' germination power because salinity produces osmotic pressure that reduces germination power. This is following the opinion of Roychoudhury (2011), who said that there is a tendency for germination speed to decrease with increasing salinity stress. The same opinion of Carpýcý (2009) stated that all germination variables for all genotypes decreased with increasing salt concentration. Ismail (1998) noted that plant responses to environmental stress, especially saline land, vary depending on the plant genotype. These differences relate to differences in tolerance of each plant genotype to stress.

From the observation data on the germination level, it was also obtained that the germination power of all genotypes was classified as low at the salinity treatment level because it was caused by the salt content in the germination media, which disrupted germination. This follows the opinion of Sembiring and Gani (2005), who stated that the effect of excessive salt on plants is a reduction in germination speed, plumule length and number of roots. In addition to low germination power, genotype resistance to salinity stress varies between genotypes. Inhibition of the root formation process is thought to be due to the accumulation of NaCl salt in the root environment.

The results of the variance analysis showed that genotypes had a very significant effect on plant height characters. It is suspected that the genotype response to salinity is very complex and varies between plant organs and stages of development, as seen in Table 5. The opinion of Kravchik.M and Bernstein. N (2013) stated that genotype responses to salinity vary and involve specificity at the organ and cell levels and variability with developmental stages and genotypes, which showed a statistically significant response to salt stress. Salinity-induced changes showed a response related to plant age from developing tissues, with an increase due to salinity stress. The genotype response to salinity reduced the number of leaves and made the leaves stunted and inhibited plant growth; this is seen in Table 5.

The results of the variance analysis also showed that the interaction between NaCl concentration and genotype at the germination level did not occur. This is thought to be because, during germination, food reserves still come from or are sourced from food reserves in the seeds, so the effect is smaller.

Germination is one of the processes of growth and development of the embryo (plant body). The result of this germination is the emergence of a small plant from within the seed. The embryo change process during germination is

when the plumule grows and develops into a stem, and the radicle grows and develops into a root. The germination process is influenced by light, temperature, and oxygen. Germination also involves physical and chemical processes.

The physical process occurs at the beginning of germination and starts at the end of the dormancy period in the seed. The end of this period is marked by the imbibition process, namely the entry of water into the seed, which causes the seed to expand and the seed coat to break. Physiologically, the germination process occurs in several critical stages, including water absorption, metabolism of the breakdown of food reserve materials, transport of materials resulting from endosperm breakdown to the actively growing embryo, re-forming new materials, respiration and growth.

A chemical process occurs when water enters the seeds. The water activates the embryo, releasing the hormone gibberellin (GA). This hormone stimulates the aleurone, the thin outer layer of the endosperm, to synthesize and secrete enzymes. These enzymes function by hydrolyzing food reserves found in the cotyledons and endosperm. For instance, the enzyme amylase converts the starch in the cotyledons into glucose, essential for energy production in the presence of oxygen. Furthermore, as growth progresses, the embryo develops into a plant.

The effect of NaCl concentration on germination is influenced by both the duration and timing of the stress. Different genotypes exhibit varying levels of Na+ content in their roots, allowing root cells to thrive under salinity stress conditions (Lauchli, 1990). During germination, seeds activate numerous metabolic processes essential for the early stages of growth. It has been observed that seeds tend to develop more robustly and perform better when subjected to stress conditions (Cramer, 2002).

4. Conclusion

The higher the NaCl concentration, the lower the germination rates, as indicated by plumule length, root length, and the number of roots. Different genotypes exhibit varying responses regarding root length, number of roots, percentage of seed growth, and overall germination rates. These variations highlight the distinct responses of genotypes to saline conditions in plant germination.

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